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A comparison of total-mixed-ration and feed-to-yield strategies on blood profiles and dairy cow health

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ABSTRACT

Seventy-two Holstein-Friesian dairy cows were offered the same amount of concentrates over the first 140-days of lactation, by either a 'total-mixed-ration' or a 'feed-to-yield' strategy. The effects on blood profiles and cow health were examined. Cows on total-mixed-ration were offered a mixed ration comprising grass silage and concentrates (50:50 dry matter basis). Cows on feed-to-yield were offered a basal mixed ration (grass silage plus 6 kg concentrates/cow/day) plus additional concentrates via an out-of-parlour feeding system, calculated according to each individual cow's milk yield during the previous week. Cows on

total-mixed-ration had a higher mean haemoglobin, packed cell volume and lymphocyte percentage. Concentrate allocation strategy had no effect on serum haptoglobin concentrations, interferon gamma production of pokeweed mitogen stimulated whole blood culture, the incidence of clinical or subclinical mastitis, lameness, respiratory or digestive problems, and no strong relationships were identified between production parameters with serum metabolites, inflammatory and immune measures. This study demonstrates small physiological differences in metabolic parameters, and no differences in inflammatory or immune parameters, when allocating concentrates by total-mixed-ration or feed-to-yield.

INTRODUCTION

Genetic selection for milk production over many decades has led to the development of today's high yielding dairy cow (Ingvartsen and Moyes, 2013). During early lactation the energy demands of these higher yielding cows normally outpace intakes (Bell, 1995), resulting in negative energy balance (EB) and adipose tissue mobilisation to meet the energy shortfall (Grummer et al., 2004; Ingvartsen, 2006). Negative EB can be detrimental to dairy cow immunity (Ingvartsen and Moyes, 2013; LeBlanc, 2014) resulting in higher incidences of diseases such as mastitis (Søndergaard et al., 2002). Multiple immune cell changes have been characterised during negative EB, including reduced neutrophil function (Kehrli et al., 1989; Burvenich et al., 2007) and reduced lymphocyte function (Nonnecke et al., 2003; Lessard et al., 2004; Lacetera et al., 2005). In addition, metabolic profiles associated with negative EB, such as low blood glucose and high non-esterified fatty acid (NEFA) and β-hydroxybutyrate (BHB) concentrations, have been shown to contribute to immune suppression in in-vitro studies (Newsholme et al., 1986; Suriyasathaporn et al., 2000; Scalia et al., 2006).

The adoption of nutritional and management strategies that reduce early lactation negative EB may reduce the risk of health problems (Ingvartsen et al., 2003; Ingvartsen, 2006). For example, once-a-day milking in early lactation has been shown to reduce serum NEFA concentrations compared with milking three times a day (Patton et al., 2006). It has also been suggested that improving dry matter (DM) intake (DMI) in early lactation to better meet energy requirements may reduce metabolic stress (Ingvartsen, 2006). Strategies by which this might be achieved include; improving volatile fatty acid (VFA) absorption capacity in the rumen thereby reducing VFA accumulation and increasing rumen pH (Dirksen et al., 1985); increasing diet digestibility and rate of passage (Ingvartsen, 2006); and offering high quality forages and increased concentrate levels (Ferris et al., 2003). It is also possible that the adoption of 'precision' feed-to-yield concentrate allocation strategies, which target concentrates to meet individual cow energy requirements (rather than a 'herd-based' total-mixed-ration approach), may reduce the extent of negative EB experienced by individual cows.

A number of studies have compared these two concentrate allocation strategies, with performance largely unaffected by allocation strategy (Taylor and Leaver, 1984a, b; Lawrence et al., 2015; Purcell et al., 2016). However, across these studies the impact of concentrate allocation strategy on cow health, blood metabolites and immune function has received little attention. To address this issue, this paper examines the metabolic, inflammatory and immune profiles, and health of dairy cows during first 140-days of lactation when allocated the same amount of concentrates by two different strategies, namely 'feed-to-yield' or as a 'total-mixed-ration'. While a detailed description of milk production, tissue changes and fertility of the cows on this study has already been presented by Little et al. (2016), the key performance parameters are summarised in this paper.

MATERIALS AND METHODS

Animals and Housing

This study involved 77 multiparous (mean parity, 3.2; SEM, 0.17; median parity 3.0) Holstein-Friesian dairy cows. Cows had a mean Predicted Transmitting Ability (PTA_{2015}) for milk yield and fat plus protein yield of 247 (SEM, 29.1) kg and 30 (SEM, 4.5) kg, respectively, and were within the top 1% of UK genetics in terms of Profitable Lifetime Index (PLI_{2014}), namely £179 (SEM, 21.1). All procedures described in this paper were conducted under an experimental license granted by the Department of Health, Social Services & Personal Safety for Northern Ireland, in compliance with the United Kingdom (UK) Animals (Scientific Procedures) Act 1986.

Throughout the experiment, cows were housed together in a free stall cubicle house with concrete flooring, which was scraped every three hours by an automated system. The cubicle to cow ratio was ≥ 1:1 at all times and cubicles were fitted with rubber mats and bedded thrice weekly with sawdust.

Experimental Design, Diets and Feeding

All cows were managed identically during the prepartum period. Within 24-hours of calving, cows were transferred from a maternity pen to the free stall cubicle house and allocated by simple randomization to one of two concentrate allocation strategies, either ‘feed-to-yield’ or ‘total-mixed-ration’. Throughout the allocation process, visual checks was made to ensure that the two treatment groups remained balanced for expected calving date, parity, previous lactation 305-day milk yield, body weight (BW) and body condition score (BCS) at previous lactation drying off, PTA for fat plus protein (kg), and number of services and calving interval during the previous lactation. With each concentrate allocation strategy, the objective

was to achieve the same total concentrate intake over a 20-week period and therefore rations were designed to be nitrogenous.

Cows on total-mixed-ration were offered a mixed ration comprising grass silage and concentrates (fixed for the study at 50:50 DM ratio). Chopped straw was included in the mix to achieve a target intake of 0.3 kg/cow/day. This ration was designed to meet maintenance energy requirements and support a mean milk yield of 40 kg/cow/day, assuming an average BW loss of 0.5 kg/cow/day, over the 20-week study. Ration formulation was based on the equations contained within 'Feed into Milk' (Agnew et al., 2004), the current UK dairy cow feed rationing system, and the metabolisable energy (ME) and intake potential of the grass silage, as determined by near-infrared reflectance spectroscopy (NIRS).

Cows on feed-to-yield were offered a basal mixed ration *ad libitum*, comprising grass silage plus concentrates, the latter included to achieve an average intake of 6.0 kg concentrate/cow/day. Chopped straw was included in the mix to achieve a target intake of 0.3 kg/cow/day. Based on the equations of Agnew et al. (2004), and the estimated intake potential and nutritive value of the silage offered, this 'basal ration' was initially estimated to sustain the maintenance energy requirements of the cow plus a milk yield of 27 kg/cow/day, which was revised to 24 kg/cow/day based on actual DMI data. In addition to this basal ration, cows on feed-to-yield were offered additional concentrates via an out-of-parlour feeding system (Fullwood Ltd, Shropshire, England). From day-1 postpartum, these additional concentrates were increased in 0.25 kg/day increments, so that cows were offered 5.25 kg/day at day-21. From day-21 onwards, concentrate feed levels were adjusted weekly based on the mean milk yield during the previous 7-day period, with concentrates offered at 0.45 kg for each kg of milk produced above what the basal diet was assumed to sustain. To

maintain efficient cow flow into the parlour, all cows on were offered an additional 0.5 kg concentrate per milking via an in-parlour feeding system.

A common concentrate was offered with both treatments. The concentrate offered mixed with the silage was in the form of a meal, while the concentrate offered via the in-parlour and out-of-parlour feeding system was in the form of a pellet. The ingredient composition of the concentrate offered (g/kg fresh basis) was as follows: maize, 215.5; wheat, 160; wheat feed, 60; sugar beet pulp, 100; distillers grains, 50; corn gluten, 40; soya hulls, 140; soya bean meal, 80; Sopralin (Trouw Nutrition, Cheshire, UK), 30; rapeseed meal, 40; lime flour, 7; salt, 7.5, calcined magnesite (Trouw Nutrition, Cheshire, UK), 3; Molaferm (United Molasses, Belfast, UK) 50; palm oil, 8; Acid buf (Celtic sea minerals, Cork, Ireland), 8; mineral/vitamin mix (Superdairy, Trouw Nutrition, Cheshire, UK), 4; Actisaf (Lesaffre, Shannon, Ireland), 0.4.

Grass silage was produced from a primary growth herbage harvested from predominantly perennial ryegrass-based swards and ensiled following a 24 to 48 hour period of field wilting. Rations were prepared using a complete diet mixer-wagon (Redrock Varicut, Redrock, County Armagh, Northern Ireland) and transferred directly to feed-boxes mounted on weigh cells. Access to treatment rations were controlled by a Calan Broadbent feeding system (American Calan Inc., Northwood, NH, USA) linked to an electronic identification system, thus enabling individual cow intakes to be recorded daily. To ensure *ad libitum* consumption, the diets for each treatment were offered at 107% of the previous day's intake. Uneaten ration was removed daily at approximately 08.00, while the fresh ration was offered between 09.00 and 10.00. Cows remained on treatments until day-140 of lactation.

Cow performance, Energy Balance and Energy Corrected Milk Yield Calculation

Milk samples were obtained once weekly from 2 consecutive milkings (am and pm), a preservative tablet added (Broad Spectrum Microtabs II, D and F Control Systems, Massachusetts, USA), and samples stored at 4°C until analyzed. These samples were analyzed weekly for fat, protein and lactose content by fourier transform infrared spectroscopy using an infrared milk analyzer (Milkoscan, model FT 120, Foss UK Ltd., Warrington, UK) and a weighted milk composition subsequently calculated for each sampling occasion. On 1 occasion each month, samples from 2 consecutive milkings, bulked in proportion to yield, were collected and SCC measured (model CA3A4, Delta Instruments, Netherlands).

All individual cow health events and treatments were recorded. Cow BW were recorded twice daily (using an automatic weighscale) and cow BCS were recorded weekly using a 1 to 5 scale (Edmonson et al., 1989) with quarter-point increments.

The mean daily ME requirements and balances for each cow were calculated using the equations of Agnew et al. (2004), where daily mean energy balance (MJ/cow per d) was determined using the equation:

$$mean\ energy\ balance\ = \left([ME_{main+milk} \times BW^{0.75}] + \left[\frac{[0.0013 \times BW]}{K_m} \right] - 10 \right) - MEi$$

where $ME_{main+milk}$ is the ME required for maintenance and milk production (MJ/kg metabolic weight), $BW^{0.75}$ is the metabolic BW, K_m is the efficiency of utilization of ME for activity (calculated as 0.35 × ME/gross energy + 0.503), and MEi is the ME intake (MJ/cow per d). Data for mean daily milk yield, milk fat, protein and lactose concentrations, and mean BW were used in the calculations for the energy balance variables. Energy corrected milk yield (ECMY) was calculated using the following formula as defined by Sjaunja et al., (1990);

ECMY (kg/cow per d) = milk yield kg × (0.383 × fat % × 0.242 × protein % + 16.54 × lactose % + 20.7) / 3.140.

Blood Measurements

Blood samples were collected at weeks 2 (11 to 17 days), 4 (25 to 31 days), 6 (39 to 45 days), 8 (53 to 59 days), 10 (67 to 73 days), 12 (81 to 87 days), 16 (109 to 115 days) and 20 (137 to 143 days) of lactation, for the measurement of albumin, BHB, glucose, glutamate dehydrogenase (GLDH), Haptoglobin (Hp), globulin, NEFA, total protein, and urea concentrations. Blood haematology and interferon gamma (IFN-γ) production from stimulated lymphocytes were assessed on a subsample of cows (17 and 20 from total-mixed-ration and feed-to-yield, respectively), with these sub-groups balanced in the same manner as previously described. Blood samples were stored and analyzed as described by Little *et al*. (2016).

Health, Somatic Cell Count and Vaginal Mucus Score Evaluation

All individual cow health events and treatments were recorded. Displaced abomasum, dilated cecum, decreased rumen motility and diarrhea were recorded as 'digestive upset'. Abnormal milk and clots were recorded as mastitis. Once a month, samples from two consecutive milkings, bulked in proportion to yield, were collected and SCC measured using mid-infrared diffuse reflectance spectroscopy (model CA3A4, Delta Instruments, Drachten, The Netherlands). Vaginal mucus was clinically scored on both smell and appearance on a 0-3 scale at weeks 2 (11 to 17 days), 3 (18 to 24 days) and 4 (25 to 31 days) postpartum, according to Little et al. (2016).

Statistical Analysis

Five cows were removed from the experiment for reasons not associated with the treatments and their data excluded from the statistical analysis, leaving 36 cows on each treatment. Data were analyzed using GenStat Version 16.2 (VSN International, Oxford, UK). Data describing somatic cell score (log^e transformed somatic cell count) were analyzed using ANOVA. Data describing blood measurements were analysed using Residual Maximum Likelihood (REML) repeated measures analysis. The mixed model used included treatment + week + treatment × week as fixed effects. Cow × week was included in the random model, to which a power model (city block metric) covariance structure was applied. Individual cow data was used to investigate relationships between EB and ECMY, and each of NEFA, BHB, glucose, WCC, neutrophil %, lymphocyte %, IFN-γ and Hp, using simple linear regression analysis. Best fit equations (either a common or different equation for both treatments) were identified. Analysis was initially undertaken using mean data for the entire study, and also using the mean data for weeks 6 to 10 postpartum, the period encompassing peak milk yield. Relationships between NEFA, BHB and glucose, and each of IFN-γ and Hp were investigated in the same way. Differences were considered to be statistically significant when $P < 0.05$. Binomial data describing health treatments were analysed using generalised linear model regression analysis with the logit link function. The model included treatment as a term and significance was identified using chi squared. Data describing vaginal mucus scores were analysed using ordinal logistic regression with the logit link function and significance was identified using chi squared. Vaginal mucus scores were translated into one integer; 0 = 0, 0; 1 = 1, 0; 2 = 2, 0; 3 = 3, 0; 4 = 2, 1; 5 = 3, 1, and for analysis, grouped into three categories, 0, 1, ≥ 2.

RESULTS

The chemical composition of the grass silage and concentrate offered is presented in Table 1. The total rations offered with the feed-to-yield and total-mixed-ration treatments had a mean ME of 11.4 and 11.4 MJ/kg DM, respectively, a mean CP of 170 and 170 g/kg DM, respectively, a mean starch content of 154 and 150 g/kg DM, respectively, and a mean NDF of 389 and 391 g/kg, respectively. A summary of a number of the main cow performance parameters, as reported previously by Little et al. (2016), is presented in Table 2. Concentrate allocation strategy had no effect ($P > 0.05$) on total DMI, concentrate DMI, milk yield, milk fat or milk protein composition, milk fat plus protein yield, mean BW, BW loss to nadir and mean EB (Table 2).

Concentrate allocation strategy had no effect ($P > 0.05$) on mean serum glucose, BHB, albumin, globulin, total protein, urea, GLDH, red cell count (RCC), white cell count (WCC), Hp or IFN-γ production (Table 3). Mean corpuscular volume (51.3 and 50.4 fL for total-mixed-ration and feed-to-yield, respectively: SED = 1.03, $P = 0.538$), mean corpuscular haemoglobin (16.5 and 16.2 pg for total-mixed-ration and feed-to-yield, respectively: SED = 0.30, $P = 0.151$), and mean corpuscular haemoglobin concentration (32.3 and 32.1 g/100mL for total-mixed-ration and feed-to-yield, respectively: SED = 0.27, $P = 0.700$), were unaffected by treatment. Cows on total-mixed-ration had a higher haemoglobin ($P = 0.009$), packed cell volume (PCV) ($P = 0.018$), and lymphocyte % ($P = 0.020$), and had a lower serum NEFA ($P = 0.028$) and neutrophil % ($P = 0.018$) than cows on feed-to-yield (Table 3). Serum NEFA ($P < 0.001$), BHB ($P < 0.001$), white cell count ($P < 0.001$) and neutrophil % ($P = 0.008$) decreased with time, while glucose ($P < 0.001$), albumin ($P = 0.002$), total protein ($P < 0.001$), urea ($P < 0.001$), GLDH ($P = 0.019$), and lymphocyte % ($P = 0.020$) increased with time (Table 3. The effect of concentrate allocation strategy on mean NEFA and BHB (Figure 1), mean blood lymphocyte and neutrophil percentage (Figure 2) and mean serum Hp and IGN- γ production (Figure 3), over the experimental period, are presented.

Significant relationships ($P < 0.05$) between EB (Table 4) and ECMY (Table 5), and biochemistry, haematology and immune parameters are presented. Using mean data over the study period, the relationship between EB and NEFA was described by separate equations for each treatment ($R^2 = 0.196$, $P < 0.001$), while the relationship between EB and WCC was described by a common equation ($R^2 = 0.258$, $P < 0.001$) for each treatment (Table 4). Using mean data for weeks 6 to 10 postpartum, the relationships between EB and NEFA ($R^2 = 0.194$, $P = 0.005$), EB and BHB ($R^2 = 0.071$, $P = 0.013$), and EB and WCC ($R^2 = 0.297$, $P < 0.001$) were described by common equations for each treatment (Table 4). Using mean data over the study period, the relationships between ECMY and NEFA ($R^2 = 0.169$, $P < 0.015$), and ECMY and BHB ($R^2 = 0.050$, $P < 0.033$) were described by common equations for each treatment, while the relationship between ECMY and WCC ($R^2 = 0.179$, $P < 0.013$) was described by a separate equation for each treatment (Table 5). Using mean data for weeks 6 to 10 postpartum, the relationships between ECMY and BHB ($R^2 = 0.178$, $P = 0.005$) and ECMY and WCC ($R^2 = 0.234$, $P < 0.001$) were described by separate equations for each treatment (Table 5). Using data for week 6 to 10 postpartum, the relationship between IFN-γ production and BHB ($r = 0.32$, $P = 0.031$) was described by a common equation.

Concentrate allocation strategy had no effect ($P > 0.05$) on the probability of obtaining different vaginal mucus scores at weeks 2, 3 and 4 postpartum (Table 6).

Concentrate allocation strategy had no effect ($P > 0.05$) on the number of cases of mastitis, lameness, respiratory problems, digestive problems or on mean Somatic Cell Scores (Table 7).

DISCUSSION

Many concentrate allocation strategies are adopted on dairy farms, from total-mixed-rations that are designed to meet the average nutrient requirements of a group of cows and rely on

DMI variation to meet nutritive requirements; to more precise feed-to-yield strategies that tailor concentrate allocations according to the milk yield of individual cows. This study was designed to examine the effects of allocating similar amounts of concentrates over a 20-week period by two very different strategies, 'feed-to-yield' and 'total-mixed-ration', on the metabolic, inflammatory and immune profiles of dairy cows. As previously reported by Little et al. (2016), cows on feed-to-yield tended to lose more BW to nadir than cows on total-mixed-ration.

Although cows on feed-to-yield had a higher NEFA than total-mixed-ration cows, the actual difference of 0.04meq/L is physiologically small. This agrees with the absence of differences between treatments in BHB and GLDH, (both of which can increase during tissue mobilization; Bell, 1995; Herdt, 2000; Otter, 2013), and the fact that treatment had no effect on mean DMI, milk yield or mean EB. Similarly, Lawrence et al. (2015) found no strong evidence for differences in tissue mobilization between cows managed on different concentrate allocation strategies. Although concentrate allocation strategy resulted in significant treatment × time interactions for concentrate DMI and milk yield (Little et al., 2016), this was not reflected in significant treatment × time interactions for the biochemistry parameters examined, which highlights that the cows were able to maintain homeorhesis (Bauman and Currie, 1980). In common with other studies (Nyman et al., 2008; Mendonça et al., 2014; McCarthy et al., 2015), NEFA declined over the study period, indicative of a decreasing rate of tissue mobilisation as lactation proceeded. The absence of a treatment × week interaction for NEFA suggests that tissue mobilisation did not differ between treatments with stage of lactation, consistent with a lack of treatment × time interactions for cow performance data (Little et al., 2016).

A key objective was to examine if individual animals within each treatment experience differences in metabolic stress. For example, with feed-to-yield, higher yielding cows are allocated additional concentrates, which tends to drive higher milk yields (Little et al., 2016) and this may cause metabolic stress. Little et al. (2016) previously demonstrated that while negative EB increased with increasing milk yields, this was more pronounced with cows on feed-to-yield. In the current study, the relationship between EB and NEFA over the study period are described by parallel lines for each treatment, indicating similar responses from cows on each treatments. However, the higher intercept with cows on feed-to-yield indicates a lesser degree of metabolic stress at higher yields compared to cows offered the total-mixed-ration. As common equations described the relationships between EB and NEFA, and EB and BHB between weeks 6 to 10 of lactation, the differences in metabolic stress between treatments was not evident during peak yield

While cows on total-mixed-ration had a higher PCV and haemoglobin compared to those on feed-to-yield, the differences are physiologically small and within normal reference ranges (Radostits et al., 2007). In addition, the absence of differences in RCC, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration suggest a negligible difference in red blood cell function between treatments. The decrease in WCC and neutrophil percentage with time from calving is consistent with the findings of others (Kehrli et al., 1989; Meglia et al., 2001), while the absence of any significant treatment × time interactions indicate that treatments did not produce a different response in these variables. There was a positive relationship between EB and WCC, and a negative relationship between ECMY and WCC. The former agrees with Morris et al. (2009), who found that a lower WCC in cows with a severe negative EB compared with those with a mild

negative EB. As these relationships are described by common equations for both treatments, concentrate allocation strategies did not affect WCC response.

The impact of concentrate allocation strategy on inflammatory and immune profiles has not previously been examined. Serum Hp was used as an indirect measure of clinical and subclinical inflammatory, infectious and metabolic conditions. That mean Hp was unaffected by treatment suggests similar levels of inflammation with both treatments. The absence of a stage of lactation effect, or a treatment x time interaction, demonstrates that the different DMI and milk yield curves for each treatment, as presented by Little et al. (2016), did not lead to differences in Hp concentrations. The absence of significant relationships between Hp concentrations and either EB or ECM, demonstrate that the extremes of concentrate intakes and milk yields with feed-to-yield did not influence the inflammatory status of individual animals. In addition, an increase in total WCC (leukocytosis) or neutrophil percentage (neutrophilia), and a decrease in lymphocyte percentage (lymphopenia), occur as part of inflammatory responses during periods of stress (Cole, 1997; Otter, 2013). Cows on feed-to-yield had a higher neutrophil percentage and lower lymphocyte percentage compared with cows on total-mixed-ration, suggestive of a degree of inflammatory stress. However, as neutrophil values remained within normal physiological ranges, these changes cannot be defined as neutrophilia, as occurs in a true stress leukogram (Radostits et al., 2007). No significant relationships were identified between either EB or ECMY, and neutrophil or lymphocyte percentages, suggesting metabolic stress or production does not affect while blood cell parameters.

Interferon gamma is a cytokine synthesised by activated T-lymphocytes, which functions to enhance immune surveillance and activate the cellular immune response during infection

(Young and Hardy, 1995; Schroder et al., 2004). Concentrate allocation strategy had no effect on the ability of lymphocytes to produce IFN-γ, while IFN-γ production did not change over time, nor was there an interaction between treatment or stage of lactation. Previous studies have shown that higher NEFA concentrations can have a suppressive effect on IFN-γ production (Lacetera et al., 2004; Ster et al., 2012), while management strategies imposed to reduce metabolic stress have been shown to mitigate the normal decrease in IFN-γ around calving (Loiselle et al., 2009). In this study, while serum NEFA were significantly lower with cows on total-mixed-ration, the magnitude of this difference (0.04 meq/L) was not sufficient to impact IFN-γ production. The lack of significant relationships between IFN-γ production and either EB or ECMY, suggests the larger range of EB and individual cow milk yields with the feed-to-yield treatment had no effect on immune function.

While most diseases in dairy cows have a multifactorial aetiology, the cow's ability to defend against infectious disease is in-part related to the efficiency of the immune system. Concentrate allocation strategy had no effect on somatic cell score or clinical mastitis incidence, suggesting that the udder defence to contagious and environmental organisms were unaffected by treatment. While the aetiology of lameness is multifactorial, with complex interactions between nutritional, metabolic, environmental and infectious risk factors (Alban, 1995; Solano et al., 2015), the efficacy of the immune response to infectious organisms can play a role in disease susceptibility (Palmer and O'Connell, 2015). Nevertheless, the current study demonstrates no impact of concentrate allocation strategy on the incidence of lameness. An effective immune response is also important in resolving the normal and unavoidable uterine bacterial contamination after calving, therefore helping to prevent against the development of clinical uterine disease (Sheldon et al., 2008; Sheldon et al., 2009). Vaginal mucus scores provided an indirect assessment of uterine bacterial infection and inflammation

(Williams et al., 2005). As there was no significant difference in vaginal mucus scores at weeks 2, 3 and 4 postpartum, this suggests that the concentrate allocation strategies did not significantly influence the immunological response to the inevitable uterine contamination in the early postpartum period, in keeping with the similar inflammatory and immune responses.

Digestive upsets in dairy cows are largely due to dietary factors rather than infectious causes. In the current study, concentrate allocation strategy had no significant effect on digestive upsets, which is in keeping with this the previous findings of no effect of concentrate allocation strategy on faecal consistency scores (Little et al., 2016),

CONCLUSION

When concentrates were allocated to dairy cows on either a feed-to-yield or a total-mixed-ration basis, health, inflammatory and immune profiles were unaffected. While concentrate allocation strategy had a number of minor effects on haematology and biochemistry profiles, these had negligible physiological consequences. No strong relationships were identified between production variables, and biochemistry, haematology, inflammatory and immune variables, and these did not greatly differ with concentrate allocation strategy.

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Table 1 Chemical composition of grass silage and concentrates offered during the study

	Grass silage	Concentrate
Oven DM (g/kg)	298	872
VCODM[1] (g/kg)	314	-
pH	3.83	-
Ammonia nitrogen (g/kg total N)	78	-
Composition of DM (g/kg)		
Crude protein	159	180
Ethanol	18.4	-
Propanol	0.8	-
Lactic acid	126	-
Acetic acid	19.2	-
Propionic acid	0.33	-
n-Butyric acid	1.04	-
I-Valeric	0.17	-
Acid detergent fiber	291	152
Neutral detergent fiber	486	303
Ash	103	73
Gross energy (MJ/kg DM)	18.3	17.9
Metabolisable energy (MJ/kg DM)	11.6[2]	11.2[3]

[1] VCODM, volatile corrected oven dry matter
[2] Predicted using Near Infrared Reflectance Spectroscopy
[3] Calculated from 'standard values'

Table 2 Summary of the main effects of concentrate allocation strategy on total dry matter intake, milk yield, milk constituents, milk constituent yield, and bodyweight, during the first 140 d of lactation

	Concentrate Allocation Strategy			
	Feed-to-yield	**Total-mixed-ration**	**SED[1]**	***P*-value**
Total dry matter intake (kg/d)	22.2	22.4	0.31	0.773
Concentrate dry matter intake (kg/d)	11.7	11.5	0.25	0.714
Milk yield (kg/d)	38.0	39.3	0.95	0.326
Milk fat (g/kg)	42.8	42.9	0.46	0.882
Milk protein (g/kg)	32.9	32.4	0.31	0.258
Fat + Protein yield (kg/d)	3.0	3.1	0.09	0.420
Mean Bodyweight (kg)	623	621	4.9	0.782
Bodyweight loss to nadir (kg)	43	33	4.2	0.088
Mean energy balance (MJ/cow per d)	-21.4	-25.5	5.26	0.426

[1] SED, standard error of the difference

Table 3 Effects of concentrate allocation strategy on serum biochemistry and blood haematology during the first 140 d of lactation (mean of samples taken at weeks 2, 4, 6, 8, 10, 12, 16 and 20 postpartum)

	Concentrate Allocation Strategy			P-value		
	Feed-to-yield	Total-mixed-ration	SED[1]	Treatment[2]	Time[3]	Treatment[2] × Time[3]
Biochemistry parameters						
Glucose (meq/L)	3.62	3.67	0.036	0.227	<0.001	0.891
NEFA (meq/L)	0.33	0.29	0.017	0.028	<0.001	0.697
BHB (mM)	0.44	0.46	0.022	0.379	<0.001	0.271
Albumin (g/L)	29.6	30.6	0.56	0.055	0.002	0.304
Globulin (g/L)	40.3	39.2	1.41	0.583	0.247	0.306
Total protein (g/L)	69.9	69.8	1.34	0.935	0.012	0.247
Urea (mM)	3.45	3.55	0.117	0.306	<0.001	0.326
GLDH (U/L)	66.1	53.8	10.34	0.444	0.019	0.064
Haematology Parameters[4]						
Red Cell Count ($x10^{12}$/L)	5.91	6.07	0.153	0.274	0.763	0.236
Haemaglobin (g/L)	94.5	99.0	1.96	0.009	0.359	0.332
Packed Cell Volume (L/L)	0.30	0.31	0.006	0.018	0.486	0.151
White Cell Count ($x10^{9}$/L)	6.61	6.45	0.322	0.859	<0.001	0.424
Lymphocyte (%)	72.7	76.3	1.68	0.020	0.005	0.571
Neutrophil (%)	24.6	21.1	1.63	0.018	0.008	0.507
Immunological parameters[4]						
Haptoglobin (mg/mL)	0.86	0.95	0.089	0.356	0.495	0.221
Interferon gamma (ng/mL)	12.9	10.6	1.087	0.115	0.959	0.455

NEFA = nonesterified fatty acid, BHB = beta-hydroxybutryrate, GLDH = glutamate dehydrogenase

[1] SED, standard error of the difference for treatment

[2] Concentrate allocation strategy

[3] Weeks postpartum

[4] Measured on a sub-group of 17 and 20 from the feed-to-yield and total-mixed-ration treatments, respectively

Table 4 Summary of significant relationships (P > 0.05) identified between energy balance and NEFA, BHB, glucose, WCC, neutrophil %, lymphocyte %, IFN-γ and Hp, for cows offered concentrates by either a feed-to-yield or total-mixed-ration strategy (mean data for the study period and for weeks 6 to 10 of lactation)

Equation	Treatment	R^2	P value
Mean data for study period			
EB = 16.95 – 115.1 NEFA	Feed-to-yield	0.196	< 0.001
EB = 8.77 – 115.1 NEFA	Total-mixed-ration		
EB = -94.1 + 10.3 WCC	[1]	0.258	< 0.001
Mean data for weeks 6 to 10 postpartum			
EB = -3.61 – 76.3 NEFA	[1]	0.094	0.005
EB = 12.2 0 – 92.3 BHB	[1]	0.071	0.013
EB = -109.20 + 11.4 WCC	[1]	0.267	< 0.001

[1] Both treatments described by a common equation

NEFA = nonesterified fatty acid, BHB = beta-hydroxybutryrate, WCC = white cell count

Table 5 Summary of significant relationships (P > 0.05) identified between energy corrected milk yield (ECMY) and NEFA, BHB, glucose, WCC, neutrophil %, lymphocyte %, IFN-γ and Hp, for cows offered concentrates by either a feed-to-yield or total-mixed-ration strategy (mean data for the study period and for weeks 6 to 10 of lactation)

Equation	Treatment	R^2	P value
Mean data for study period			
ECMY = 34.66 + 16.46 NEFA	[1]	0.069	0.015
ECMY = 33.55 + 13.96 BHB	[1]	0.050	0.033
ECMY = 53.20 – 2.10 WCC	Feed-to-yield	0.179	0.013
ECMY = 47.12 – 0.72 WCC	Total-mixed-ration		
Mean data for weeks 6 to 10 postpartum			
ECMY = 20.81 + 51.7 BHB	Feed-to-yield	0.178	<0.001
ECMY = 33.33 + 19.23 BHB	Total-mixed-ration		
ECMY = 58.93 – 2.39 WCC	[1]	0.234	0.001

[1] Each treatment described by separate equations

NEFA = nonesterified fatty acid, BHB = beta-hydroxybutryrate, WCC = white cell count

Table 6 Effects of concentrate allocation strategy on the probability (and standard error) of obtaining a mucus score of 0, 1, ≥ 2 on wk 2, 3, and 4 (± 3 d) of lactation

	Mucus score			P value
	0	**1**	**≥ 2**	
Week 2				
Feed-to-yield	0.19 (0.150)	0.40 (0.136)	0.40 (0.131)	
Total-mixed-ration	0.09 (0.171)	0.30 (0.138)	0.61 (0.107)	0.080
Week 3				
Feed-to-yield	0.39 (0.141)	0.34 (0.143)	0.26 (0.156)	
Total-mixed-ration	0.54 (0.114)	0.30 (0.144)	0.17 (0.152)	0.199
Week 4				
Feed-to-yield	0.63 (0.105)	0.28 (0.150)	0.08 (0.159)	
Total-mixed-ration	0.59 (0.110)	0.31 (0.140)	0.10 (0.170)	0.725

Table 7 Effects of concentrate allocation strategy on the percentage of cows treated for a number of health problems, and on somatic cell count and somatic cell score during the first 140 d of lactation

	Concentrate Allocation Strategy			
	Feed-to-yield	**Total-mixed-ration**	**SED[1]**	**P-value**
Percentage of cows treated for:[3]				
Clinical Mastitis	31	36	7.8	0.617
Lameness	28	14	6.6	0.144
Respiratory problems	14	8	5.2	0.451
Digestive disorders	22	8	5.8	0.096
Somatic Cell Count (1000/mL)	91	61		
Somatic Cell Score[2]	10.82	10.45	0.23	0.125

[1] SED, standard error of the difference for treatment

[2] Natural logarithm ($\log_e$) of somatic cell count

[3]Percentage of cows with at least one incidence